Differential effects of Ca^{2+} –calmodulin on adenylate cyclase activity in mouse and rat pancreatic islets

Peter THAMS, Kirsten CAPITO and Carl J. HEDESKOV

Department of Biochemistry A, University of Copenhagen, 3C Blegdamsvej, DK-2200 Copenhagen N, Denmark

(Received 22 February 1982/Accepted 5 March 1982)

The effects of Ca^{2+} –calmodulin on adenylate cyclase activity in EGTA-washed, 27000g particulate fractions of mouse and rat pancreatic islets were studied. Ca^{2+} ($10\mu\text{M}$)–calmodulin ($1\mu\text{M}$) stimulated adenylate cyclase activity 53.1 ± 5.2 ($n = 6$)% in the particulate fraction of rat islets. Trifluoperazine ($50\mu\text{M}$), a specific inhibitor of calmodulin, inhibited the Ca^{2+} –calmodulin activation of the adenylate cyclase activity of this fraction of rat islets. These results confirm previous reports dealing with Ca^{2+} –calmodulin and rat islet adenylate cyclase [Valverde, Vandermeers, Anjaneyulu & Malaisse (1979) *Science* **206**, 225–227; Sharp, Wiedenkeller, Kaelin, Siegel & Wollheim (1980) *Diabetes* **29**, 74–77]. In contrast, however, Ca^{2+} (1 – $100\mu\text{M}$)–calmodulin (1 – $10\mu\text{M}$) did not stimulate the adenylate cyclase activity in the EGTA-washed particulate fraction of mouse islets, and trifluoperazine ($50\mu\text{M}$) did not inhibit the adenylate cyclase activity of this fraction of mouse islets, although some remaining calmodulin [0.18 ± 0.05 ($n = 3$) $\mu\text{g}/\text{mg}$ of protein] could be demonstrated. GTP ($10\mu\text{M}$) enhanced islet adenylate cyclase activity considerably, but did not confer any sensitivity towards Ca^{2+} –calmodulin on mouse islet adenylate cyclase. The results question the role of calmodulin in the Ca^{2+} -dependent rise in cyclic AMP evoked by glucose in pancreatic islets.

A rise in the intracellular concentration of Ca^{2+} is believed to trigger insulin release by the pancreatic β -cell (Hedekov, 1980). The mediator of the regulatory effects of Ca^{2+} on insulin release may be the Ca^{2+} -dependent regulator calmodulin. Thus pancreatic islets were recently reported to contain calmodulin (Sugden *et al.*, 1979) and trifluoperazine, a presumed specific antagonist of calmodulin function, has been observed to inhibit glucose-stimulated insulin secretion (Gagliardino *et al.*, 1980; Valverde *et al.*, 1981).

Cyclic AMP has been shown to have a marked potentiating effect on the ability of glucose to stimulate insulin release from islets (Hedekov, 1980). In addition, intracellular concentrations of cyclic AMP can be modestly increased by stimulatory levels of glucose (Charles *et al.*, 1973; Capito & Hedekov, 1974). The mechanism by which glucose increases intracellular cyclic AMP is not understood.

Abbreviations used: Tes, 2-[[2-hydroxy-1-1, bis-(hydroxymethyl)ethyl]amino]ethanesulphonic acid; NTA, nitrilotriacetic acid; IBMX, 3-isobutyl-1-methylxanthine.

Some evidence suggests that the raised intracellular Ca^{2+} concentration in response to glucose is responsible for stimulation of the adenylate cyclase. Thus, as previously demonstrated in rat and *ob/ob* mouse islets, the effect of glucose in raising cyclic AMP levels depends on the presence of extracellular Ca^{2+} (Charles *et al.*, 1975; Hellman, 1976). Furthermore, the capacity of a number of other agents, e.g. glyceraldehyde, ionophore A23187 and *p*-chloromercuribenzenesulphonate, to increase and of somatostatin to decrease cyclic AMP levels in islets seems to be correlated to the ability of these agents to affect the concentration of Ca^{2+} in islet cells (Hedekov, 1980). Ca^{2+} in itself (10 – $100\mu\text{M}$) has been observed to inhibit adenylate cyclase activity in mouse islet homogenates (Capito & Hedekov, 1977). However, Ca^{2+} stimulation of adenylate cyclase as observed in brain tissue has been found to be mediated by calmodulin (Brostrom *et al.*, 1975), which may not be present in sufficient concentrations when the enzyme is studied in the broken-cell situation.

Recently, it has been reported that Ca^{2+} –calmodulin stimulates rat islet adenylate cyclase activity

(Valverde *et al.*, 1979; Sharp *et al.*, 1980). Consequently, it was suggested that the glucose-induced accumulation of cyclic AMP in islets is a secondary consequence of the rise in cytosolic Ca^{2+} , which, with calmodulin, causes an increase in the activity of adenylate cyclase (Valverde *et al.*, 1979; Sharp *et al.*, 1980).

In a previous report from this laboratory (Capito & Hedekov, 1977) glucose was found to stimulate the adenylate cyclase activity in homogenates of mouse islets. Contrary to the effect of glucose in intact islets, this effect was not dependent on added Ca^{2+} .

In seeking an explanation for the ability of glucose to raise cyclic AMP levels in islets, this study aimed at investigating the possible involvement of Ca^{2+} -calmodulin in the regulation of islet adenylate cyclase activity. The results confirm that Ca^{2+} -calmodulin stimulates rat islet adenylate cyclase activity. However, it is also demonstrated that Ca^{2+} -calmodulin does not affect mouse islet adenylate cyclase activity.

Experimental

Materials

Crude bacterial collagenase, creatine kinase, phosphocreatine (disodium salt), cyclic AMP and GTP (dilithium salt) were obtained from Boehringer, Mannheim, Germany. Tes, EGTA and ATP (disodium salt; A2383) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. NTA was obtained from E. Merck, Darmstadt, Germany. IBMX was from EGA-Chemie, Steinheim/Albuch, Germany. Trifluoperazine was a gift from Rhône-Poulenc Pharma Norden A/S, Denmark. Human serum albumin was from Behringwerke A.G., Marburg, Germany. [^3H]Cyclic AMP (sp. radioactivity 27 Ci/mmol) was from The Radiochemical Centre, Amersham, Bucks., U.K. Calmodulin was prepared from bovine pancreas (Vandermeers *et al.*, 1977). The prepared calmodulin was homogeneous as judged by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Calmodulin-deficient phosphodiesterase was prepared from bovine heart (Sharma & Wang, 1979). All other chemicals were of analytical grade, obtained from ordinary commercial sources.

Preparation of islets

Islets were prepared by using collagenase digestion (Coll-Garcia & Gill, 1969) from the pancreases of 200 g male albino rats or 26 g male albino mice fed *ad libitum* on a standard laboratory diet.

Preparation of islet particulate fractions

A group of 350 rat islets or 1000 mouse islets were collected in 700 μl of 25 mM-Tes buffer, pH 7.60, containing 5 mM- MgCl_2 and 1 mM-EGTA. A homogenate was prepared by sonication (10 s, 40 W). The homogenate was centrifuged for 20 min at 27000 g at 4°C. The pellet was resuspended and centrifuged twice more in the same volume of the same Tes buffer. After the last centrifugation and resuspension, the preparation was frozen in liquid N_2 and stored at -80°C until the next day. Protein was measured with bovine serum albumin as standard as described by Groves *et al.* (1968).

Assay of adenylate cyclase

The reaction mixture (80 μl) contained 3 mM-ATP, 5 mM- Mg^{2+} , 0–100 μM - Ca^{2+} , 1 mM-IBMX, 0.15% human serum albumin, 20 mM-NTA, 1 mM-EGTA, 25 mM-Tes buffer, pH 7.60, 5 mM-phosphocreatine, 0.5 mg of creatine kinase/ml, 3–4 μg of rat or mouse islet particulate protein, and further additions as indicated in Fig. 1 and the Tables. Mg^{2+} and Ca^{2+} were added as the chlorides. The reaction was carried out at 30°C for 60 min and terminated by heating at 95°C for 3 min. After cooling, 60 μl of ZnSO_4 (0.4 M) and 60 μl of Na_2CO_3 (0.4 M) were added for precipitation of ATP. After centrifugation at 6000 g for 10 min, the supernatants were stored at -20°C until assay for cyclic AMP. The activity was proportional to the amount of islet particulate protein and was linear with time for at least 75 min. Cyclic AMP was measured by a competitive protein-binding assay (Geisler *et al.*, 1977).

Assay of calmodulin

Concentrations of calmodulin in heat-denatured samples were determined by recombination with calmodulin-dependent cyclic nucleotide phosphodiesterase. Briefly, a sample in 25 mM-Tes buffer, pH 7.60, containing 5 mM- MgCl_2 and 1 mM-EGTA was heated at 95°C for 3 min. After cooling, an equal volume of the same Tes buffer also containing 0.2% human serum albumin was added to the sample. The sample was then assayed for calmodulin in a reaction mixture (90 μl) containing 40 mM-Tris/HCl, 40 mM-imidazole/HCl, 5 mM- MgCl_2 , 0.2 mM- CaCl_2 , calmodulin-dependent phosphodiesterase, 10 μl of the heated sample and 1.2 mM- [^3H]cyclic AMP (4.6 Ci/mol) at pH 7.50. The reaction was carried out at 30°C for 30 min. At the end of the incubation period 10 μl of a solution containing 10 mM-cyclic AMP, 10 mM-AMP and 10 mM-adenosine was added and the reaction was stopped by addition of 25 μl of 10% trichloroacetic acid. In all experiments, the amount of enzyme added caused hydrolysis of less than 30% of the substrate. After centrifugation at 6000 g for 5 min, trichloroacetic acid in a 100 μl portion was

extracted by 100 μ l of a 0.5 M-tri-n-octylamine solution in trichlorotrifluoroethane (Chen *et al.*, 1977). The separation and determination of ³H-labelled nucleotides by chromatography on polyethyleneimine-cellulose thin-layer sheets were achieved by the method described by Torp-Pedersen *et al.* (1979).

Calculations

Ionized concentrations of bivalent metal ions in the adenylate cyclase assay were calculated by using a computer program (Flodgaard & Fleron, 1974). This program takes into account all known values of pH, ionic strength, acid dissociation constants and stability constants of possible metal-ion complexes (Sillen & Martell, 1971; Smith & Alberty, 1956). In all experiments the concentration of Mg²⁺ was calculated to be 5 mM. Statistical evaluation of the data was made by a paired *t*-test. Results are given as means $\pm$ S.E.M. (*n* = number of experiments).

Results

Effects of Ca²⁺ and calmodulin on adenylate cyclase activity

Ca²⁺-calmodulin stimulated the adenylate cyclase activity in an EGTA-washed particulate fraction derived from rat islets (Fig. 1a). The stimulation by calmodulin was dependent on Ca²⁺ and biphasic (activation then inhibition) with increasing Ca²⁺ concentrations with maximum stimulation of approx. 50% above controls at 10 μ M-Ca²⁺ (*P* < 0.001). At 10 μ M-Ca²⁺ the calmodulin dependence of the activity exhibited saturation kinetics with an apparent *K_m* for calmodulin close to 0.1 μ M.

Calmodulin at 1 μ M was the maximum stimulating concentration (results not shown). Optimum stimulation by Ca²⁺-calmodulin was obtained at 10 μ M-Ca²⁺ irrespective of the calmodulin concentration used (0.05–10 μ M) (results not shown). Ca²⁺ alone exerted a slightly stimulatory effect at 1–20 μ M-Ca²⁺, the stimulation at 1 μ M being approx. 10% greater than controls (*P* < 0.02). Ca²⁺ 100 μ M was inhibitory (*P* < 0.01) (Fig. 1a).

In contrast with these results, Ca²⁺-calmodulin did not stimulate the adenylate cyclase activity in an EGTA-washed particulate fraction derived from mouse islets over the range 1–100 μ M-Ca²⁺ (Fig. 1b). Calmodulin alone did not affect the activity of the mouse preparation either. Furthermore, higher concentrations of calmodulin (up to 10 μ M) did not reveal any stimulation in mouse islets either in the absence or in the presence of 1–100 μ M-Ca²⁺ (results not shown). Ca²⁺ alone slightly stimulated adenylate cyclase activity at 1 μ M, the stimulation being approx. 10% greater than controls (*P* < 0.001).

Glucose (16.7 mM) in the absence or in the presence of Ca²⁺ did not stimulate adenylate cyclase activity in particulate fractions or whole homogenates of mouse islets (results not shown).

Effect of trifluoperazine

Mouse pancreatic islets were found to contain 0.58 ± 0.05 (*n* = 3) ng of calmodulin per islet or 0.70 ± 0.06 (3) μ g of calmodulin per mg of protein. Of this, approx. 7% or 0.18 ± 0.05 (3) μ g of calmodulin per mg of protein was found to be present in the EGTA-washed particulate fraction of mouse islets. In comparison, rat islets were found to contain 1.46 ng of calmodulin per islet or 2.28 μ g of

Fig. 1. Dose-action relationship of the effect of Ca²⁺ on islet adenylate cyclase activity with (▲) and without (○) exogenous calmodulin (1 μ M)

Islet adenylate cyclase activity was determined as described in the Experimental section. (a) Rat islet particulate fraction; (b) mouse islet particulate fraction. Results are means $\pm$ S.E.M. (*n* = 5–6) and are percentages of the control value [784 ± 70 (*n* = 6) pmol of cyclic AMP/60 min per mg of rat islet protein and 719 ± 35 (6) pmol of cyclic AMP/60 min per mg of mouse islet protein; means $\pm$ S.E.M.]. Each experiment comprised all the given Ca²⁺ concentrations with and without calmodulin.

calmodulin per mg of protein (means of two determinations). Approx. 0.15 μg of calmodulin per mg of protein was found to be present in the particulate fraction of rat islets (mean of two determinations). Although these amounts of calmodulin in the particulate fractions only correspond to approx. 0.4 nM-calmodulin in the adenylate cyclase assay, the possibility existed, that the adenylate cyclase derived from mouse islets could already be fully activated by residual membrane-bound Ca^{2+} -calmodulin. However, trifluoperazine, presumed to be a specific inhibitor of calmodulin functions through binding to the Ca^{2+} -calmodulin complex (Levin & Weiss, 1977), did not inhibit basal adenylate cyclase activity in either mouse or rat particulate fractions in the presence or in the absence of $10\text{ }\mu\text{M}$ - Ca^{2+} (Table 1). Furthermore, trifluoperazine (10 – $50\text{ }\mu\text{M}$) did not prevent the slightly stimulatory effect of $1\text{ }\mu\text{M}$ - Ca^{2+} in the mouse preparation (results not given). Trifluoperazine did, however, inhibit the Ca^{2+} -calmodulin-stimulated adenylate cyclase activity in the rat preparation (Table 1).

Effects of Ca^{2+} and calmodulin on GTP-activated adenylate cyclase activity

GTP has been found to increase the affinity of Ca^{2+} -calmodulin to brain adenylate cyclase (Brostrom *et al.*, 1978), and it has been suggested that the guanine nucleotide-binding regulatory protein of adenylate cyclase is required for Ca^{2+} -calmodulin stimulation of adenylate cyclase (Toscano *et al.*, 1979). GTP ($10\text{ }\mu\text{M}$) stimulated both mouse [74.5 ± 6.5 (5)% above controls] and rat [130.8 ± 7.8 (6)% above controls] islet adenylate cyclase activity in EGTA-washed particulate fractions. GTP ($10\text{ }\mu\text{M}$) did not, however, confer any sensitivity towards Ca^{2+} -calmodulin upon mouse islet adenylate cyclase at $1\text{ }\mu\text{M}$ -calmodulin, either at $10\text{ }\mu\text{M}$ - Ca^{2+} (Table 2) or at $1\text{ }\mu\text{M}$ - Ca^{2+} (not shown). Furthermore, GTP ($10\text{ }\mu\text{M}$) failed to elicit any stimulation by Ca^{2+} -calmodulin of the mouse enzyme in the presence of $10\text{ }\mu\text{M}$ - Ca^{2+} and $10\text{ }\mu\text{M}$ -calmodulin (results not shown).

The stimulatory effect of Ca^{2+} ($10\text{ }\mu\text{M}$)-calmodulin ($1\text{ }\mu\text{M}$) on the rat enzyme was not significantly

Table 1. *Effect of trifluoperazine on adenylate cyclase activity in particulate fractions of rat and mouse pancreatic islets*
Islet adenylate cyclase activity was determined as described in the Experimental section. Results are mean percentages $\pm$ S.E.M. of the control value [784 ± 70 ($n = 6$) pmol of cyclic AMP/60 min per mg of rat islet protein and 719 ± 35 (6) pmol of cyclic AMP/60 min per mg of mouse islet protein; means $\pm$ S.E.M.]. Statistical evaluation of the data was made by a paired *t* test. Abbreviations: NS, not significant ($P > 0.05$); TFP, trifluoperazine.

Added agents	Rat particulate fraction			Mouse particulate fraction		
	Activity (% of control)	<i>n</i>	<i>P</i> (versus control)	Activity (% of control)	<i>n</i>	<i>P</i> (versus control)
Nil	100			100		
Calmodulin ($1\text{ }\mu\text{M}$)	102.3 ± 3.5	6	NS	101.3 ± 2.9	6	NS
Ca^{2+} ($10\text{ }\mu\text{M}$)	108.5 ± 2.3	6	<0.02	100.3 ± 4.4	6	NS
Ca^{2+} ($10\text{ }\mu\text{M}$) + calmodulin ($1\text{ }\mu\text{M}$)	153.1 ± 5.2	6	<0.001	99.8 ± 3.1	6	NS
TFP ($10\text{ }\mu\text{M}$)	98.2 ± 3.3	5	NS	91.3 ± 5.2	5	NS
TFP ($10\text{ }\mu\text{M}$) + Ca^{2+} ($10\text{ }\mu\text{M}$)	104.9 ± 4.6	5	NS	84.6 ± 5.7	5	NS
TFP ($50\text{ }\mu\text{M}$)	92.1 ± 4.3	5	NS	103.8 ± 4.3	5	NS
TFP ($50\text{ }\mu\text{M}$) + Ca^{2+} ($10\text{ }\mu\text{M}$)	97.0 ± 6.8	5	NS	92.2 ± 8.8	5	NS
TFP ($50\text{ }\mu\text{M}$) + Ca^{2+} ($10\text{ }\mu\text{M}$) + calmodulin ($1\text{ }\mu\text{M}$)	116.2 ± 3.0	4	<0.02	98.7 ± 12.2	4	NS

Table 2. *Effects of Ca^{2+} and calmodulin on GTP-stimulated adenylate cyclase activity in particulate fractions of rat and mouse pancreatic islets*

Islet adenylate cyclase activity was determined as described in the Experimental section. Results are given as mean percentages $\pm$ S.E.M. of the GTP-stimulated adenylate cyclase activity [1803 ± 161 ($n = 6$) pmol of cyclic AMP/60 min per mg of rat islet protein and 1293 ± 54 (5) pmol of cyclic AMP/60 min per mg of mouse islet protein; means $\pm$ S.E.M.]. Statistical evaluation of the data was made by a paired *t* test. Abbreviation: NS, not significant ($P > 0.05$).

Added agents	Rat particulate fraction			Mouse particulate fraction		
	Activity (%)	<i>n</i>	<i>P</i> *	Activity (%)	<i>n</i>	<i>P</i> *
GTP ($10\text{ }\mu\text{M}$)	100			100		
GTP ($10\text{ }\mu\text{M}$) + calmodulin ($1\text{ }\mu\text{M}$)	102.7 ± 4.9	4	NS	107.7 ± 2.3	4	<0.05
GTP ($10\text{ }\mu\text{M}$) + Ca^{2+} ($10\text{ }\mu\text{M}$)	103.5 ± 4.3	4	NS	93.4 ± 3.4	4	NS
GTP ($10\text{ }\mu\text{M}$) + Ca^{2+} ($10\text{ }\mu\text{M}$) + calmodulin ($1\text{ }\mu\text{M}$)	134.0 ± 10.0	4	<0.05	103.1 ± 2.9	4	NS

* Versus GTP-stimulated activity.

affected by the presence of GTP. Thus the activity due to Ca²⁺ (10 μ M)-calmodulin (1 μ M) was 53.1 ± 5.2 (6)% of controls in the absence of GTP; and in the presence of GTP the activity due to Ca²⁺ (10 μ M)-calmodulin (1 μ M) was 88 ± 24.2 (6)% of controls without GTP. The lack of a potentiating effect of GTP probably reflects the fact that 1 μ M-calmodulin was the maximum stimulating concentration for rat adenylate cyclase in the absence of GTP, since GTP has been observed to affect the sensitivity, but not the maximum response, to Ca²⁺-calmodulin (Brostrom *et al.*, 1978).

Discussion

The present findings confirm that Ca²⁺-calmodulin stimulates adenylate cyclase activity in a particulate fraction of rat islets, a stimulation that previously has been suggested to provide an explanation for the glucose-induced rise in islet cyclic AMP levels (Valverde *et al.*, 1979; Sharp *et al.*, 1980). Thus the calmodulin-dependent adenylate cyclase in rat islets responds in a biphasic manner (activation then inhibition) to increasing Ca²⁺ concentrations with maximum stimulation at approx. 10 μ M-Ca²⁺ in the presence of 1 μ M-calmodulin. Since the concentration of calmodulin in intact rat islets is estimated to be about 30–40 μ M (see below), this finding supports the concept that a rise in the cytosolic concentration of Ca²⁺ by glucose may lead to the activation of adenylate cyclase by endogenous calmodulin. However, the lack of evidence for a Ca²⁺-calmodulin-dependent adenylate cyclase in mouse pancreatic islets as demonstrated in the present study questions this role of calmodulin.

The possibility that a Ca²⁺-calmodulin stimulation of mouse islet adenylate cyclase has been overlooked under the experimental conditions used seems unlikely. First, the present findings for rat islet adenylate cyclase agree with the well-documented characteristics for Ca²⁺-calmodulin stimulation of brain adenylate cyclase (Brostrom *et al.*, 1978). This agreement includes both the biphasic stimulation by Ca²⁺-calmodulin with increasing Ca²⁺ concentrations and the demonstration of simple saturation kinetics for the calmodulin dependence of the enzyme activity in the presence of Ca²⁺ with an apparent K_m for calmodulin close to 0.1 μ M-calmodulin at 10 μ M-Ca²⁺. Secondly, a stimulation by Ca²⁺-calmodulin of the adenylate cyclase in whole homogenates of mouse islets could not be demonstrated (results not shown). Thirdly, trifluoperazine did not inhibit the mouse enzyme in the absence or in the presence of Ca²⁺. Fourthly, GTP, which has been observed to increase the affinity of Ca²⁺-calmodulin for brain adenylate cyclase 3-fold (Brostrom *et al.*, 1978), failed to elicit any stimu-

lation by Ca²⁺-calmodulin of mouse islet adenylate cyclase. Finally, both the mouse and rat enzyme were stimulated by glucagon (not shown) and GTP, indicating that both enzyme preparations were functionally intact. On the other hand, it cannot be excluded that some factor distinct from calmodulin but important for the expression of Ca²⁺-calmodulin-dependent adenylate cyclase activity in mouse islets is not available in sufficient concentrations when the enzyme is studied in the broken-cell situation.

The observed species variation may not be surprising, since rat islets are composed of approx. 60% β -cells and 40% non- β -cells (Hellerström *et al.*, 1964), whereas mouse islets consist of approx. 80% β -cells and only 20% non- β -cells (Brolin & Hellerström, 1967). Thus, the relatively small (50%) increase in adenylate cyclase activity seen with Ca²⁺ and calmodulin in rat islets may originate from their greater content of non- β -cells.

In agreement with previous reports (Sugden *et al.*, 1979; Valverde *et al.*, 1979) rat islets were found to contain approx. 1.5 ng of calmodulin per islet. Assuming equal distribution of calmodulin in the intracellular water space and a total water space of approx. 2–3 nl/islet (Ashcroft *et al.*, 1970), this amount corresponds to 30–40 μ M-calmodulin in rat islets. In comparison with rat islets, the concentration of calmodulin in mouse islets appears to be somewhat lower. Mouse islets were found to contain approx. 0.6 ng of calmodulin per islet, which with the assumptions above corresponds to 12–16 μ M-calmodulin in mouse islets.

In mouse islets 1 μ M-Ca²⁺ stimulated the adenylate cyclase activity 10% above controls and higher concentrations of Ca²⁺ did not affect the enzyme activity. These findings are at variance with previous results from this laboratory (Capito & Hedekov, 1977), which demonstrated an inhibitory action of Ca²⁺ over the range 10–100 μ M-Ca²⁺ in homogenates of mouse pancreatic islets. This discrepancy in inhibition pattern may reflect the well known interaction of Ca²⁺ with Mg²⁺-sensitive sites of adenylate cyclase (Drummond & Duncan, 1970), since the concentration of ionized Mg²⁺ was changed from 1.22 mM in the previous report to 5 mM in the present study. Furthermore, it has been shown lately that the buffer capacity for Ca²⁺ of EDTA, which was used in the previous experiments, is insufficient over the range 0.01–10 μ M-Ca²⁺ (Torp-Pedersen, 1981). Consequently, the slightly stimulatory effect of 1 μ M-Ca²⁺ observed in the present experiments may have been masked by the marked inhibitory action of Ca²⁺ in the previous report. Neither the stimulatory effect of 1 μ M-Ca²⁺ (mouse islets) nor the inhibitory effect of 100 μ M-Ca²⁺ (rat islets) could be prevented by trifluoperazine (results not shown). Although the physio-

logical significance is unclear, a role for calmodulin as a mediator of these effects thus seems unlikely.

We have previously reported a direct glucose stimulation of adenylate cyclase in a homogenate of mouse pancreatic islets (Capito & Hedekso, 1977). This observation could not be reproduced in the present experiments. The reason for this discrepancy may be due to the differences in the assay medium, since we are still able to demonstrate a glucose effect in the medium originally described. However, in spite of this we have for several reasons chosen to use the assay medium described in the present paper. Studies of calmodulin effects demand a very precise control of Ca^{2+} and, as described above, the Tris/EDTA buffer previously used does not allow a satisfactory determination of the concentration of Ca^{2+} in the assay. In addition, we have recently found that glucose is metabolized even faster by whole homogenates than by intact islets both in the present and in the previously used adenylate cyclase medium (measured as formation of $^3\text{H}_2\text{O}$ from $[5\text{-}^3\text{H}]\text{glucose}$). It thus no longer seems possible to draw firm conclusions concerning the effects of glucose itself or glucose metabolites on adenylate cyclase activity if a homogenate of islets is used.

We do not know yet to what extent glucose and glucose metabolites are metabolized in the presence of the particulate fraction used in the present experiments, but we hope that the use of this preparation will eventually lead to a demonstration of which glucose metabolite, if any, is responsible for the glucose-stimulated accumulation of cyclic AMP that can be observed in intact islets.

This work was supported by financial aid from the Danish Medical Research Council (Grant no. 12-2201), NOVO's Fond, Nordisk Insulinfond, Dr. med. Erik Garde og Elizabeth Garde's legat, and the Danish Diabetic Association. The skilful technical assistance of Mrs. Ingrid Mikkelsen is highly appreciated.

References

- Ashcroft, S. J. H., Hedekso, C. J. & Randle, P. J. (1970) *Biochem. J.* **118**, 143–154
- Brolin, S. E. & Hellerström, C. (1967) *Opusc. Med.* **12**, 261–272
- Brostrom, C. O., Huang, Y.-C., Breckenridge, B. M. & Wolff, D. J. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 64–68
- Brostrom, M. A., Brostrom, C. O. & Wolff, D. J. (1978) *Arch. Biochem. Biophys.* **191**, 341–350
- Capito, K. & Hedekso, C. J. (1974) *Biochem. J.* **142**, 653–658
- Capito, K. & Hedekso, C. J. (1977) *Biochem. J.* **162**, 569–573
- Charles, M. A., Fanska, R., Schmid, F. G., Forsham, P. H. & Grodsky, G. M. (1973) *Science* **179**, 569–571
- Charles, M. A., Lawecki, J., Pictet, R. & Grodsky, G. M. (1975) *J. Biol. Chem.* **250**, 6134–6140
- Chen, S.-C., Brown, P. R. & Rosie, D. M. (1977) *J. Chromatogr. Sci.* **15**, 218–221
- Coll-Garcia, E. & Gill, J. R. (1969) *Diabetologia* **5**, 61–66
- Drummond, G. I. & Duncan, L. (1970) *J. Biol. Chem.* **245**, 976–983
- Flodgaard, H. & Fleron, P. (1974) *J. Biol. Chem.* **249**, 3465–3474
- Gagliardino, J. J., Harrison, D. E., Christie, M. R., Gagliardino, E. E. & Ashcroft, S. J. H. (1980) *Biochem. J.* **192**, 919–927
- Geisler, A., Klysner, R., Thams, P. & Christensen, S. (1977) *Acta Pharmacol. Toxicol.* **40**, 356–368
- Groves, W. E., Davis, F. C. & Milner, B. H. (1968) *Anal. Biochem.* **22**, 195–210
- Hedekso, C. J. (1980) *Physiol. Rev.* **60**, 442–509
- Hellerström, C., Hellman, B., Petersson, B. & Alm, G. (1964) in *The Structure and Metabolism of the Pancreatic Islets* (Brolin, S. E., Hellman, B. & Knutsson, H., eds.), pp. 117–129, Pergamon, Oxford
- Hellman, B. (1976) *Experientia* **32**, 155–157
- Levin, R. M. & Weiss, B. (1977) *Mol. Pharmacol.* **13**, 690–697
- Sharma, R. K. & Wang, J. H. (1979) *Adv. Cyclic Nucleotide Res.* **10**, 187–198
- Sharp, G. W. G., Wiedenkeller, D. E., Kaelin, D., Siegel, E. G. & Wollheim, C. B. (1980) *Diabetes* **29**, 74–77
- Sillen, L. G. & Martell, A. E. (1971) *Chem. Soc. Spec. Publ.* **25**, 235–807
- Smith, R. M. & Alberty, R. A. (1956) *J. Am. Chem. Soc.* **78**, 2376–2380
- Sugden, M. C., Christie, M. R. & Ashcroft, S. J. H. (1979) *FEBS Lett.* **105**, 95–100
- Torp-Pedersen, C. (1981) Ph.D. Thesis, University of Copenhagen
- Torp-Pedersen, C., Treiman, M. & Thorn, N. A. (1979) *J. Neurochem.* **32**, 1085–1091
- Toscano, W. A. Jr., Westcott, K. R., LaPorte, D. C. & Storm, D. R. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5582–5586
- Valverde, I., Vandermeers, A., Anjaneyulu, R. & Malaisse, W. J. (1979) *Science* **206**, 225–227
- Valverde, I., Sener, A., Lebrun, P., Herschuelz, A. & Malaisse, W. J. (1981) *Endocrinology* **108**, 1305–1312
- Vandermeers, A., Vandermeers-Piret, M.-C., Rathé, J., Kutzner, R., Delforge, A. & Christophe, J. (1977) *Eur. J. Biochem.* **81**, 379–386